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12 a. DISTRIBUTION / AVAILABILITY STATEMENT

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12 b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

We collected migratory white-crowned sparrows and demonstrated for the first time in the laboratory setting that during migration, birds maintain a high level of performance as demonstrated by normal to increased levels of responding on operant tasks, whereas sleep-deprived birds show decreased responding. We have completed the first continuous EEG recordings in a set of 8 birds during migration and have found that they reduce their total daily sleep by as much as 90% for periods of days to weeks at a time during migration; these data establish the migratory bird as the only known natural model that meets the goals of the Continuous Assisted Performance initiative. In addition, we have developed the technical capability to perform prolonged sleep deprivations and record and analyze bird sleep in an automated fashion. We have successfully performed the first prolonged sleep deprivations in birds and demonstrated that they show effects similar to those seen in sleep deprived mammals, further validating the bird as a model with relevance to humans. Finally, we have identified a small number of genes that appear to be specific for migratory sleeplessness, providing a group of potential targets for drug development.

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Summary of the most important results

Abstract

We studied two avian models, migratory sleeplessness and unihemispheric sleep, to identify mechanisms that allow birds to suspend temporarily the need for sleep while maintaining cognitive and physical performance. During Phase 1, we collected migratory white-crowned sparrows and demonstrated for the first time in the laboratory setting that during migration, birds maintain a high level of performance as demonstrated by normal to increased levels of responding on operant tasks, whereas sleep-deprived birds show decreased responding. Through EEG recordings in 8 birds during migration, we found a reduction in total daily sleep by up to 90% for periods of days to weeks at a time during migration; these data establish the migratory bird as the only known natural model that meets the goals of the CAP initiative. In addition, we have developed the technical capability to perform prolonged sleep deprivations and record and analyze bird sleep in an automated fashion. We demonstrated in prolonged sleep deprivations that birds show effects similar to those seen in sleep deprived mammals, further validating the bird as a model with relevance to humans. Finally, we have identified genes that appear to be specific for migratory sleeplessness, providing a group of potential targets for drug development.

Scientific Progress and Accomplishments

During Phase 1, we focused on Task 2, the migratory sleeplessness model, and essentially completed all of our goals for the project period. We also made significant progress on Task 1, although this was slowed somewhat by the departure of several technicians for graduate school and our inability to hire replacements without a continued source of funding.

Task 1 (Unihemispheric Sleep)

Behavioral and Neurophysiological studies: We performed prolonged total sleep deprivation in pigeons and have completed deprivations for up to 30 days in a total of six pigeons. Considerable time has been spent modifying the computer scoring algorithm for bird sleep, since muscle activity is not as reliable for distinguishing sleep-wakefulness states in birds as it is in mammals. Although muscle activity per se is unreliable we observed that movement (including fine movement) was a good indicator of sleep/wake state. We then used video motion detection and routed the output of the motion detection into our sleep detection program allowing us to set motion thresholds and to more accurately and reliably distinguish between sleep and wakefulness. We also began to use this method on sparrows (see Task 2 below.)

Results from the most recent sleep deprivation trials have confirmed our preliminary findings previously reported. First, we have additional evidence indicating that sleep deprived birds display increased energy expenditure and associated changes in body temperature, key features of the sleep deprivation syndrome observed in mammals <Figure 1 - Energy and Temperature>. Moreover, as in mammals, REM sleep showed an almost 100% increase in the deprived birds and only a slight increase in the yoked control <Figure 2 - Recovery Change>. This difference in recovery between deprived and control birds confirms that the disk-over-water is in fact

having a differential effect on sleep while administering the same amount of stimulation to the deprived and control birds. More importantly, these results show that avian sleep architecture is affected by sleep deprivation in the same way as mammals.

Task 2 (Migratory Sleeplessness)

We performed continuous EEG sleep recordings on eight sparrows, beginning in early August just before they commenced the fall migration. In addition to manually scoring records, we began work on developing a semi-automated scoring system for sleep scoring by computer. We have developed and performed the first validation pass of a scoring system based on the raw electrophysiological recordings. We are working on the system to extend it to the filtered recordings. It is now capable of reducing the amount of time spent in manual scoring and should soon be capable of assessing sleep well enough to allow for near instantaneous testing of measures that putatively reduce the need for sleep.

Scoring of the sleep EEG demonstrated a marked decrease in sleep during migration <Figure 3 – Changes in Sleep during Fall Migration>. The birds decreased their daily sleep amount to about 1/3 of normal during migration. Although birds were getting some sleep during fall migration, this is consistent with reports in the literature of fall migration being less intense than the spring migration. The seasonal difference in migration intensity suggests that migratory songbirds have the capacity to markedly reduce sleep as well as to virtually dispense with sleep altogether. Our EEG recordings also demonstrate that when the birds are exhibiting migratory restlessness (i.e., whirring their wings), there is no indication of unihemispheric sleep in the EEG; the birds are both behaviorally and electrophysiologically entirely awake. To summarize, these are the first continuous EEG recordings ever performed on birds during the state of migration and document that birds dramatically reduce their total daily sleep, and that they are not sleeping unihemispherically.

To compare migratory sleeplessness with comparable amounts of sleep deprivation, and to be ready to test the efficacy measures for enhancing continuous performance, it was necessary to develop a method to produce prolonged sleep deprivation that controls for the methods used to produce deprivation. Since we have been successful in producing prolonged avian sleep deprivation using the disk-over-water for pigeons, we adapted this device for perching birds <Figure 4 - POW>. The resulting perch-over-water involves a set of radial perches, like spokes on a wheel, that can be rotated to arouse the bird. We have built a perch-over-water apparatus and have begun testing its ability to produce prolonged sleep deprivation.

We have also continued cognitive testing using the Repeated Acquisition Task (i.e., three-response chain on a FR 3 schedule) in groups of sparrows from both the migratory and non-migratory subspecies. These birds have been performing the task for almost 1 and 1/2 years. Over the last two migrations, a clearer pattern of differences between the two subspecies during the migratory periods has emerged. The migratory birds, while migrating, show significantly enhanced performance <Figure 5 - Operant Responding> without decrement in accuracy <Figure 6 - Response Accuracy> in comparison to the non-migratory subspecies studied at the same. They also show a higher level of performance during migration than during the non-migratory season. These data document for the first time that migration does not interfere in any way with

performance, and that in fact during periods of prolonged sleeplessness birds can sustain higher than average levels of physical and cognitive performance, thus providing an excellent model for the warfighter who needs to engage in sustained operations.

Using mRNA differential display (mRNA DD), we have completed an extensive screening of gene expression in the cerebral cortex of groups of migratory and non-migratory subspecies of sparrows, during both migratory and non-migratory seasons (n=6 birds/group; total of 24). To date, we have run a total of 91 primer combinations and analyzed 50-100 genes per gel, thus screening up to 9,000 genes. Despite studying wild-caught birds from two different species, there has been a very high degree of consistency in gene expression across all birds and across the two seasons. Specifically, we have identified two species-specific transcripts and 7 seasonal transcripts out of the 9,000 screened so far. The high level of consistency is in agreement with our previous mRNA DD results in rats, and strongly suggests that the experimental design we used is properly controlled for technical variability (i.e. variability due to methodological inconsistencies during brain dissection, RNA extraction, reverse transcription, and PCR amplification) as well as for biological variability (i.e. variability due to individual inter- and intra-specific differences). Most important for the CAP program, we have identified a total of 7 transcripts whose expression is specifically modulated by migration in the migratory subspecies only, including 3 transcripts upregulated during migration and 4 transcripts upregulated during the non-migratory state. Figure 7 <Figure 7 - gel> shows a post-migratory transcript identified over the last 3 months. Because of the high consistency in the expression of the great majority of the transcripts screened so far, the migration- and post-migration-related transcripts are of great interest and their future characterization will yield a small number of potential target genes to be pursued in Phase 2.

In summary, the migratory white-crowned sparrow has proven to be a unique model for prolonged sleeplessness without physical or cognitive impairment; it is the only known natural model for the goals of the CAP program. We have identified a small number of genes that appear to be specific for migratory sleeplessness, providing a group of potential targets for drug development. We hope to continue recording them through the completion of their fall migration and into the post-migratory phase to be able to assess whether there is any evidence of rebound or recovery sleep following migration.

Everything worked according to plan; we did not have any substantial technical difficulties or negative results. Overall, our accomplishments in phase 1 have given us a strong basis to go into phase 2 with the realistic expectation of developing countermeasures to reverse the effects of prolonged sleeplessness in humans.

Figure 1. Energy and Temperature

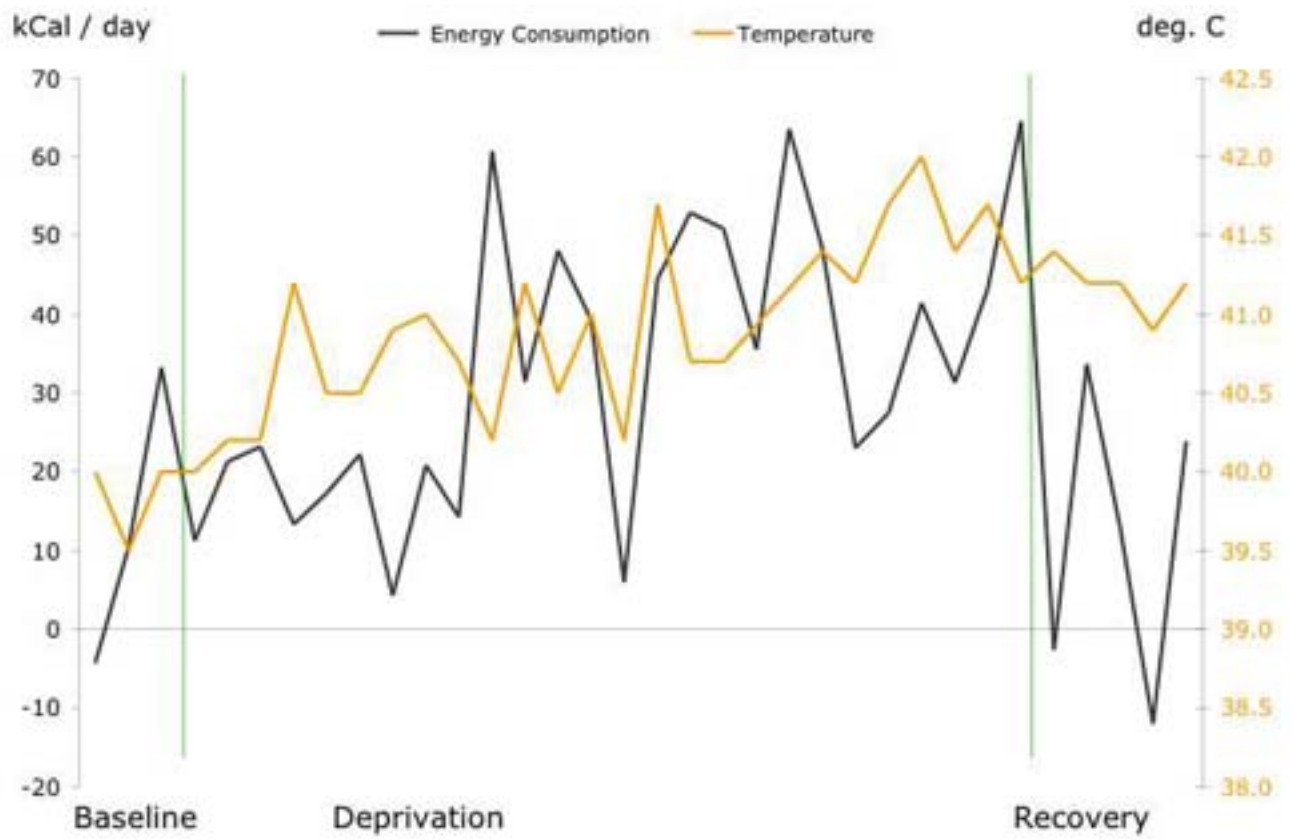


Figure 2. Recovery Change

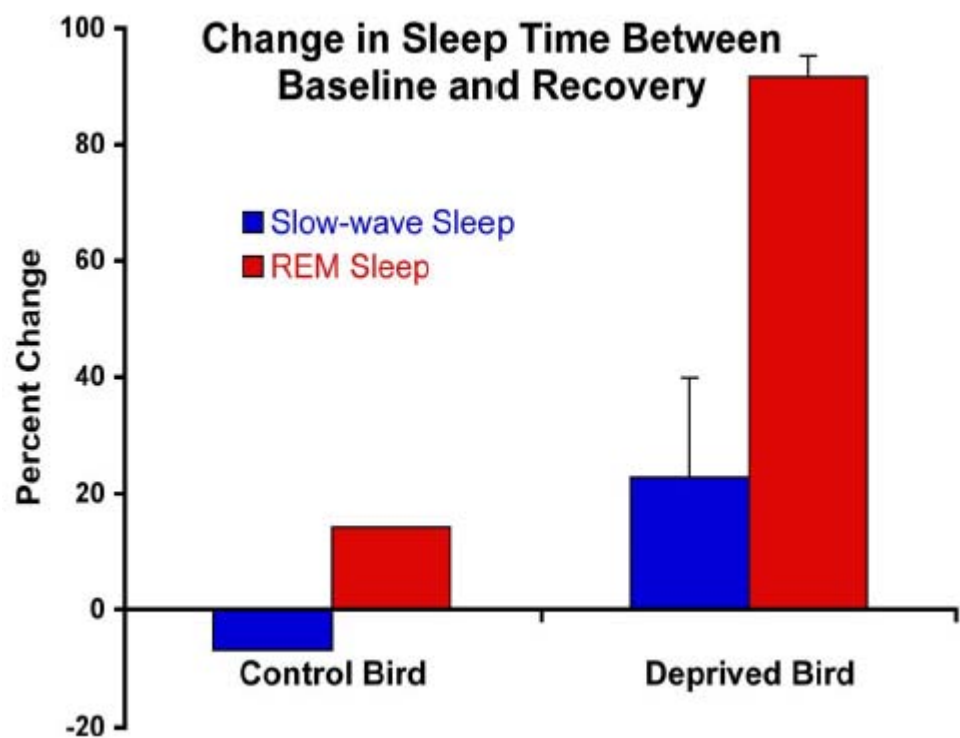
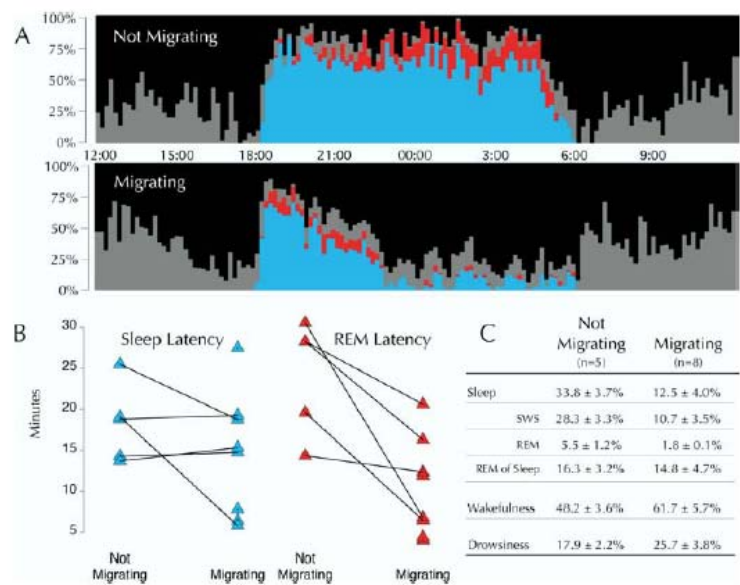


Figure 3. Changes in Sleep during Fall Migration



tory state; average sleep latency did not differ significantly between nonmigrating and migrating birds. REM sleep latency was calculated as the length of time from sleep onset to the first occurrence of REM sleep. Note that REM sleep occurred earlier in sleep during migration for all five birds that were recorded in both a nonmigratory and migratory state ($t = 3.3$, paired, two-tailed, $p < 0.05$). Note also that the REM sleep latencies for the three birds recorded only in a migratory state were shorter than the shortest REM sleep latency in nonmigrating birds.

(C) Sleep percentages. Average daily percentages of sleep and wakefulness states for birds in a nonmigrating ($n = 5$) and migrating ($n = 8$) state. Total sleep is the sum of SWS and REM sleep. For all states of vigilance, values for the migrating condition differed significantly from the nonmigrating condition ($p < 0.01$, after Bonferroni correction). The proportion of total sleep occupied by REM sleep was not significantly different between migratory states.

Figure 3. Changes in Sleep during Fall Migration

Behavioral state was scored across 24-h (noon to noon) periods using a combination of video and electrophysiological recordings for birds in a nonmigratory ($n = 5$) and migratory ($n = 8$) state. The plots and table reflect the average for all birds in each group. All recordings were performed under a 12:12 LD photoperiod with lights turned off at 18:00 and on at 06:00.

(A) Proportion of time in each behavioral state for nonmigrating (top) and migrating (bottom) birds. The proportion of every 10-min period spent in each sleep/wakefulness state was calculated for each bird and then averaged across all birds: wakefulness (black), drowsiness (gray), SWS (blue), and REM sleep (red). Note that overall sleep propensity in migrating birds is greatly diminished between approximately 22:30 and 06:00. Note also the increased propensity for REM sleep from 18:00 to 20:00 as compared to the same time period when not migrating.

(B) Sleep and REM sleep latencies. Sleep latency was calculated as the length of time from lights out until the first occurrence of sleep (in all cases SWS) for birds in a nonmigratory and migratory

Figure 4. Perch-Over-Water

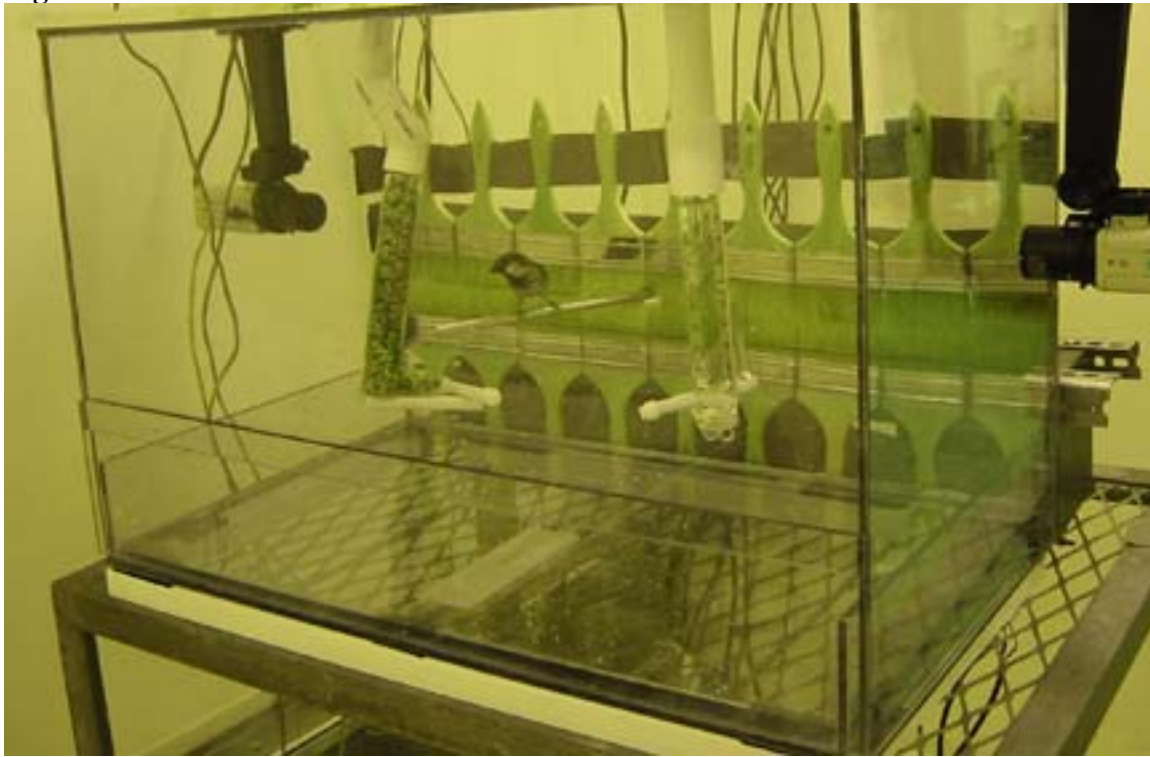


Figure 5. Operant Responding

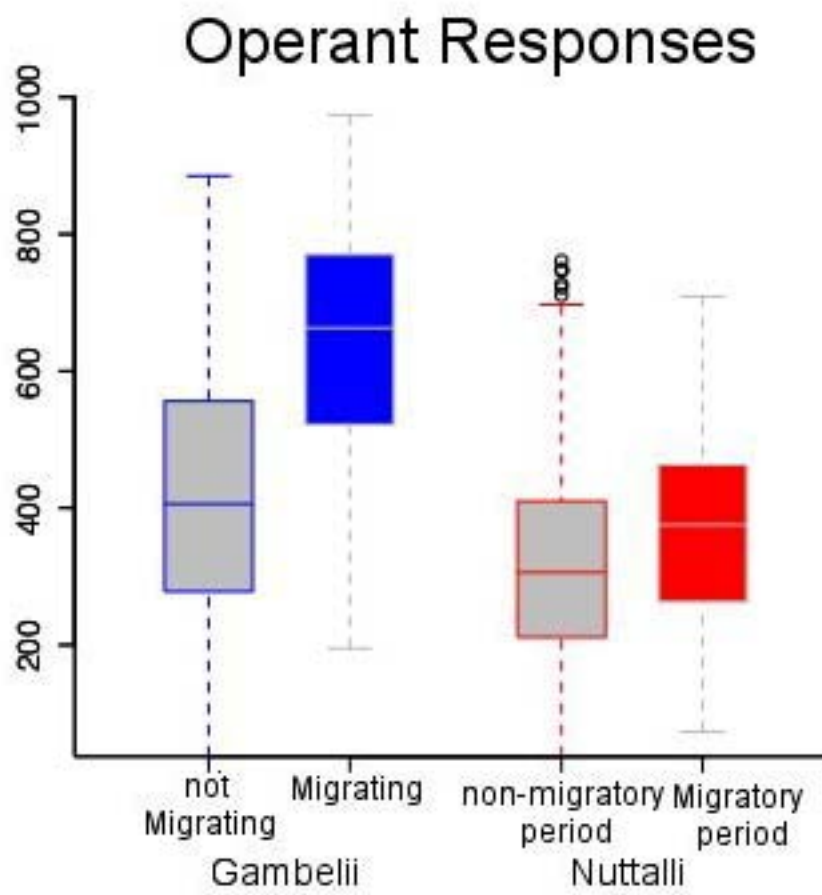


Figure 6. Response Accuracy

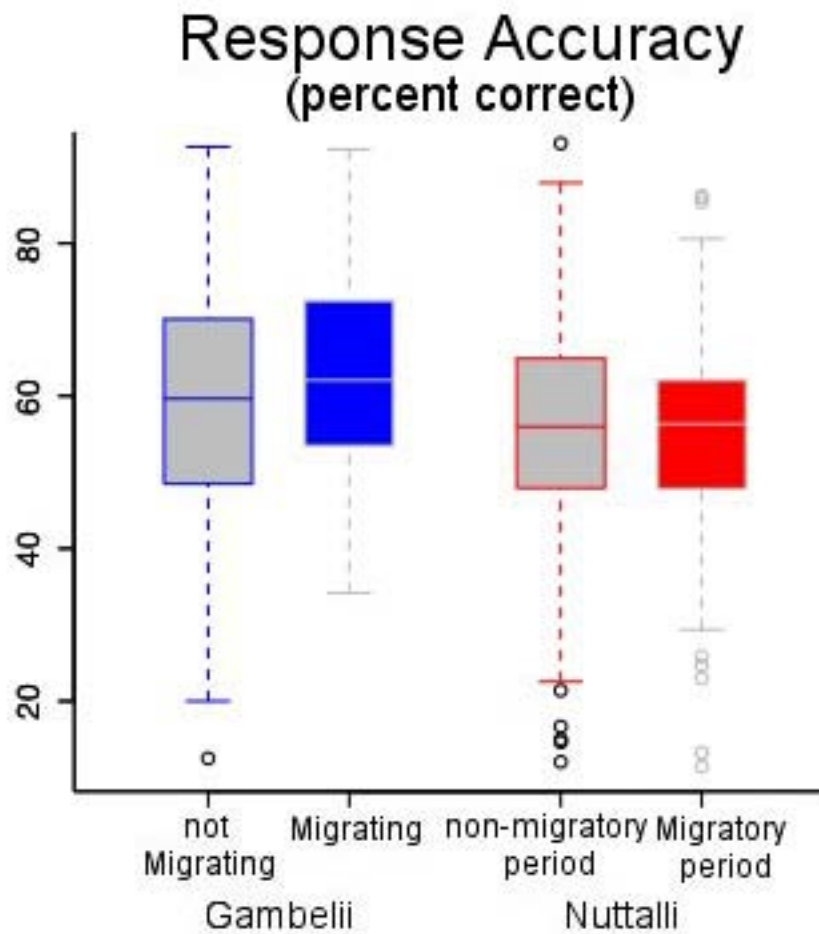
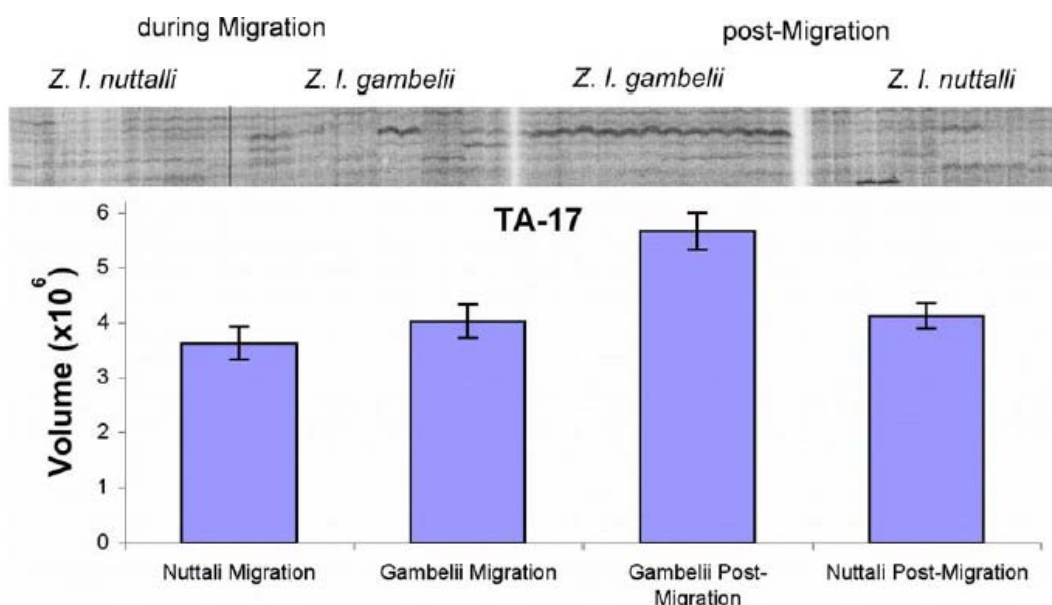


Figure 7. Gel



Listing of all publications and technical report supported under this grant

a. Papers published in peer-reviewed journals

Martinez-Gonzalez D, Obermeyer W, Fahy JL, Riboh M, Benca RM. REM sleep deprivation induces changes in coping responses that are not reversed by amphetamine. *Sleep*. 2004 Jun 15;27(4):609-17.

Rattenborg NC, Mandt BH, Obermeyer WH, Winsauer PJ, Huber R, Wikelski M, Benca RM. Migratory sleeplessness in the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *PLoS Biol*. 2004 Jul;2(7):E212.

b. Papers published in non-peer reviewed journals or in conference proceedings

Sleep, Vol 26 Abstract Supplement, 2003. (Also presented at APSS 2003 in Chicago, IL):

#0288.F: Rattenborg NC, Mandt B, Uttech R, Newman SM, Jones, S, Wikelski M, Obermeyer W, Benca RM. "Migratory Sleeplessness in the white-crowned sparrow (*Zonotrichia leucophrys gambelii*)."

#0484.I: Rattenborg NC, Obermeyer WH, Benca RM. "Sleep deprivation in the pigeon (*Columba livia*) by the disk-over-water method."

Sleep, Vol 27 Abstract Supplement, 2004. (Also presented at APSS 2004 in Philadelphia, PA):

#182: Rattenborg N, Mandt B, Wikelski M, Obermeyer W, Benca R. "Electrophysiological correlates of migratory sleeplessness in the white-crowned sparrow (*Zonotrichia leucophrys gambelii*)."

#352: Mandt BH, Rattenborg NC, Winsauer PJ, Thalasin MN, Obermeyer WH, Benca RM. "Effects of migration on cognitive performance in the white-crowned sparrow (*Zonotrichia leucophrys gambelii* & *Zonotrichia leucophrys nuttali*)."

#357: Newman S, Rattenborg N, Obermeyer WH, Benca RM. "Effects of sleep deprivation by the disk-over-water method in pigeons."

#862: Jones S, Rattenborg N, Cirelli C, Benca R. "Molecular correlates of migratory sleeplessness in the white-crowned sparrow."

c. Papers presented at meetings, but not published in conference proceedings

Oral presentation. APSS 2004 in Philadelphia, P: Martinez-Gonzalez D, Fahy J, Obermeyer W, Benca R. "Amphetamine does not reverse sleep deprivation-induced deficits in defensive responses."

d. Manuscripts submitted, but not published

N/A

e. Technical reports submitted to ARO
N/A

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Report of Inventions

No inventions resulted from this project.